



Immerge

Storming
Immune
Monogenic
Conditions
through
Multiomic and
Gene Editing
Approaches



Funded by
the European Union

12 full-time Doctoral Candidates positions

HOST INSTITUTIONS:

- **Fundació Institut de Recerca Contra la Leucemia Josep Carreras (IJC-CERCA). Spain > Badalona**
- **Centre National de la Recherche Scientifique (CNRS). France > Marseille**
- **Universitätsklinikum Freiburg (UKFRL). Germany > Freiburg**
- **Ospedale San Raffaele SRL (OSR). Italy > Milan**
- **European Molecular Biology Laboratory Heidelberg (EMBL). Germany > Heidelberg**
- **Research Fund of the Hadassah Medical Organisation (R.A.) (RFHMO). Israel > Jerusalem**
- **Quantitative Genomics Medicine Laboratories SL (Qgenomics). Spain > Barcelona**
- **Alia Therapeutics srl. Italy > Trento**
- **Fondazione per l'Istituto di Ricerca in Biomedicina (IRB). Switzerland > Bellinzona**
- **Genome Research Limited (GRL) (SANGER Institute). The UK > Cambridge**

OPEN CALL: 2 October 2023

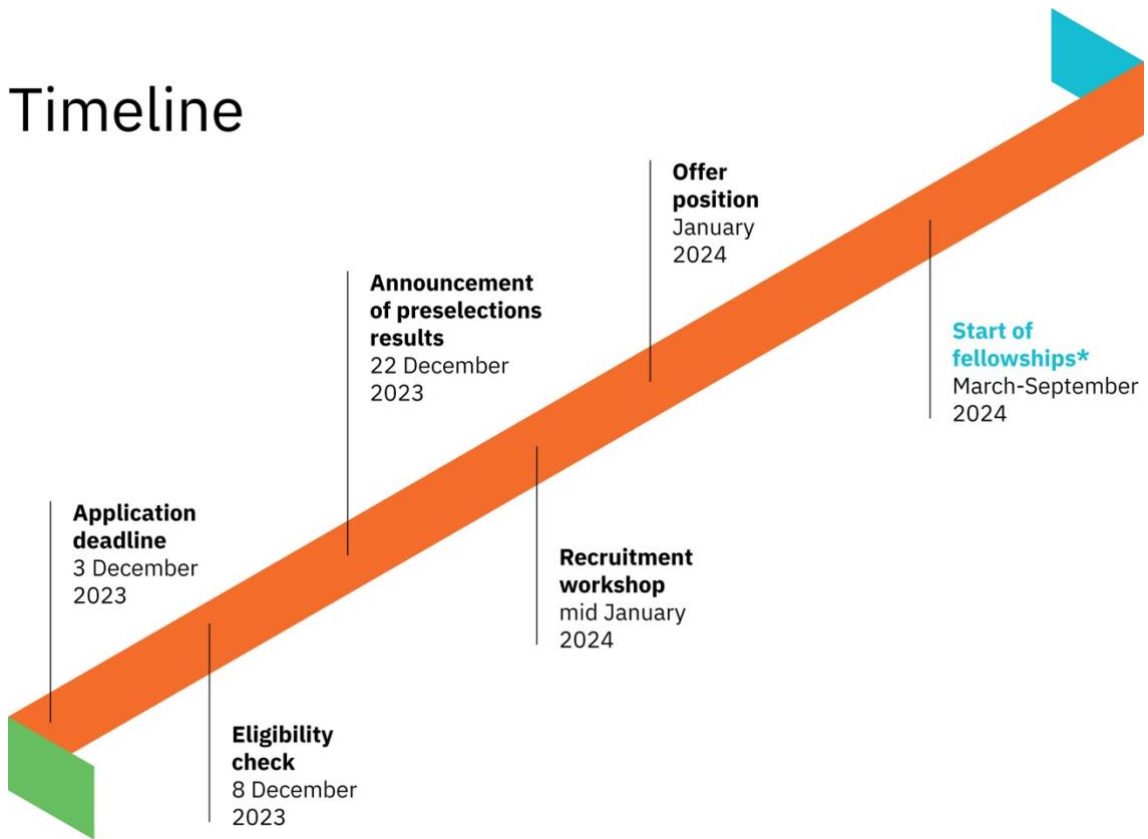
APPLICATION DEADLINE: 3 December 2023 12.00 PM

EU RESEARCH FRAMEWORK PROGRAMME: HORIZON EUROPE

MARIE SKOŁODOWSKA CURIE ACTIONS DOCTORAL NETWORKS

GRANT AGREEMENT NUMBER: 101119927

Timeline



About IMMERGE

In the realm of cutting-edge research and academic excellence, the **IMMERGE MSCA Doctoral Network (DN)** stands as an extraordinary training initiative, embracing a multidisciplinary perspective that spans various domains of knowledge. The program is dedicated to recruit 12 remarkable Doctoral Candidates (DCs) within the field of Immunology, Epigenetics, Omics and Gene Editing Technologies and Bioinformatics. The objective of the program is to answer several essential questions regarding the Inborn Errors of Immunity (IEI), such what is the relationship between genetic mutations and the wide clinical phenotypic expressivity and drug response in different individuals? How are the specific effects of these mutations modulated by the genetic background and/or environmental factors? What is the interplay between these mutations and a wealth of cell signalling pathways and transcriptional factors? Can we correct them? Inborn Errors of Immunity (IEI) represent a paradigm for (i) exploring the communication between genetics, epigenetic and environmental determinants and (ii) testing the potential of gene editing methods. The DCs will be enrolled in top-notch academic and non-academic partners working with experts in immunology, genetics, epigenetics, proteomics, single cell omics, bioinformatics, and gene correction. Participating institutions represent 7 different countries, with 8 members in academia and 2 non-academic, from the biotech sector. In addition, the overall programme has designed a tailored training programme in which 16 Associated Partners bring additional expertise to the MSCA DN.

Individual research projects

Doctoral Candidate 1: Understanding the functional & epigenetic impact of NF- κ B mutants in dendritic cell/macrophage differentiation.

Rationale and Objectives: Mutations in different transcription factors and cell signalling molecules account for a significant proportion of IEI. Defects in NF- κ B activation lead to a broad range of developmental manifestations and infections due to impaired signalling pathways downstream of both innate and adaptive immune system receptors. DC1's project will focus on the analysis of the impact in the immunological properties in relation to the epigenomic and transcriptomic changes related to altered immune responses in different cell types during differentiation and activation in individuals carrying different NF- κ B pathway mutations. Specific aims for DC1's project are: 1) To characterize the impact of selected mutations related to the NF- κ B pathway in the ability to differentiate and activate dendritic cells/macrophages derived from monocytes, monitored by flow cytometry, ELISA, etc. 2) To profile DNA methylation, selected histone modifications, transcriptome and cytokine profiling in monocytes differentiated to dendritic cells/macrophages carrying NF- κ B-pathway mutations. Characterization of the interplay between TFs and epigenetic enzymes. 3) To genetic and pharmacologically modulate the immunogenic properties of monocyte-derived dendritic cells isolated from patients with different NF- κ B-pathway mutations and determine the impact on epigenomic and transcriptomic profiles.

Host Institution: Fundació Institut de Recerca Contra la Leucèmia Josep Carreras

Degree awarding institution: University of Barcelona (UB), Spain

Supervisor: Dr. Esteban Ballestar

Planned secondment(s): CCI (Grimbacher) to receive training on various immunological methods, m10-m13 (3 months); GRL (Vento-Tormo) to receive training on standards for single-cell omics analysis methods, m15-m18 (1 month); IRB (Geiger) to receive training on gene editing, m21-m24 (3 months). Finally, DC1 will learn how to convert the output from his/her findings into epigenetic detection kits by doing a secondment at EpiQMax. m26-m28 (2 months).

Doctoral Candidate 2: Exploring TET-mediated epigenetic control in NF- κ B mutated patients.

Rationale and objectives: Mutations in NF- κ B subunits encoding-genes result in a common variable immunodeficiency (CVID) phenotype with recurrent infections and autoimmunity. However, CVID penetrance in NF- κ B mutated patients is incomplete, thus suggesting the involvement of yet unknown mechanisms including epigenetics, as recently shown by the Ballestar's and the Vento-Tormo's groups (Rodriguez-Ubreva et al., Nat Comms. 2022). TET enzymes are needed for balanced blood cell differentiation towards lymphoid and myeloid lineages (Lazarenkov and Sardina. Cancers (Basel). 2022). Recent work by the Ballestar's and Sardina's groups has uncovered mechanism linking TET2-mediated active DNA demethylation with NF- κ B activity indicating cooperativity between both mechanisms in mounting immune responses (Morante-Palacios. Nucleic Acids Res. 2022). Our hypothesis is the CVID phenotype onset in *NFKB*-mutated patients might be triggered by TET-mediated epigenomic rewiring caused by physiological aging of the immune system. Such hidden phenotype has been observed in TET2-mutated HSPCs developing myeloid malignancies only when exposed to inflammatory cues (Zhang et al., Nature. 2015; Meisel et al., Nature. 2018). Specific DC2's aims include: 1) To perform genome-wide profiling of TET-mediated 5hmC mark in B and T cell populations harboring different NFKB genetic variants. 2) To assess TET2 chromatin binding on B and T cell populations isolated harboring different NFKB genetic variants. 3) If genetic variants of interest are unavailable within the consortium cohort, to introduce them by CRISPR/Cas9 in HSPCs and T cell progenitors (from donors) and differentiate them into the B and T cells subsets of interest. 4) To utilize the NFKB-mutated cellular models to perturb TET activity and study chromatin changes and cellular differentiation capacity towards the lymphoid lineage.

Host Institution: Fundació Institut de Recerca Contra la Leucèmia Josep Carreras

Degree awarding institution: University of Barcelona (UB), Spain

Supervisor: Dr. José Luis Sardina

Planned secondment(s): SRF (Di Micco) to receive training on standards for gene correction, m10-m13 (2 months). IRB (Geiger) to learn CRISPR screens, m13-m16 (2 months); EMBL (Zaugg), to receive training on standards for genome-wide data analysis, m16-m17 (1 month).

Finally, DC2 will learn how to convert the output from his/her gene editing experiments by doing secondments at Alia m22-m24 (2 months) and at OneChain m27-m28 (1 month).

Doctoral Candidate 3: Understanding the functional impact of eif2ak mutants in dendritic cell and Interferonopathies.

Rationale and Objectives: The cellular Integrated Stress Response (ISR) mechanism reduces protein synthesis in response to stress, while establishing a transcriptional program favoring stress resolution and cell survival through the activation of EIF2A kinases. Upon immune system unbalance, Plasmacytoid dendritic cells (pDC), monocytes and B cells can fuel auto-immunity by abnormally releasing cytokines and type-I Interferon (IFN) that contribute to disease recurrence. We showed that molecules in the cellular integrated stress response (ISR), such as PERK (EIF2AK3) are required to produce type-I IFN in response to nucleic acids (NA) or toxins, key events in Interferonopathies onset and flairs. We have identified, in collaboration with the F. Rieux-Laucat (IHU Imagine, Paris), new rare human variants with increased susceptibility to familial Systemic Lupus Erythematosus (SLE) and STING-associated vasculopathy in infancy (SAVI) patients displaying mutations in various eif2ak genes, including eif2ak3/perk. We will focus on pDC, that display ISR-like features and produce recurrently type-I IFN during interferonopathies. DC3 will therefore: 1) Study how ISR induction in pDC leads type-I IFN production, which becomes pathogenic in susceptible individuals bearing mutations in eif2ak genes. Cell biology methodologies, as well as protein synthesis, energy metabolism and cytokines monitoring by advanced flow cytometry will be performed on Cas9 engineered differentiated HSCs and transformed cell models, as well as on PBMCs. 2) Identify the gene/mutation-specific omics signatures in mutated patient or engineered model cell lines. Ribo-seq analysis and proximity biotinylation identification (BIO-ID) by mass spectrometry will be performed to reveal the molecular networks linking different ISR molecular players to the innate immunity signaling pathways, like the anti-viral STING adaptor and ultimately type-I IFN production and/or activation of the JAK/STAT pathways. 3) Characterize using cell biology and immunology approaches the crosstalk between microbe or toxin sensing and the ISR in pDCs the potentially contributes to interferonopathies onset by potentializing the responses in mutated cells.

Host institution: Centre National de la Recherche Scientifique

Degree awarding institution: Aix-Marseille Université (AMU), France

Supervisor: Dr. Philippe Pierre

Planned secondment(s): IJC (Ballestar) and OSR (Di Micco) to learn in vitro differentiation protocols of immune cells and gene inactivation, m10-m13 (3months); GRL (Vento-Tormo) to learn multi-omics data analysis, (m18-20) and Alia to learn about the gene editing procedures developed in industry, m22-24 (2 months).

Doctoral Candidate 4: Diving deep into the biology of STAT3 regulation and -signaling

Rationale and Objectives: For patients with loss-of-function mutations in STAT3 the only currently available cure is hematopoietic stem cell transplantation (HSCT). However, HSCT comes along with a considerable mortality during treatment, especially in adults (expected to be around 20%). Therefore, gene therapy has been suggested as a possible alternative. One option for gene therapy is to inactivate the mutated allele, which however renders the patient STAT3 haplo(in)sufficient. Another approach is to replace both STAT3 alleles with a cassette for STAT3-cDNA expression, eliminating however, alternative STAT3 transcripts. STAT3 (encoding the signal transducer and activator of transcription-3) represents a paradigm of the highly complex JAK-STAT signal transduction network, which comprises four Janus kinases, seven STAT molecules and additional splice variants. The transcription factor STAT3 is involved in both, the pro-inflammatory IL-6 pathway and the anti-inflammatory IL-10 signaling. Although intensively investigated, the mechanisms by which STAT3 signaling differentiates between pro-and anti-inflammatory signals, remains enigmatic. STAT3 is the lineage-defining transcription factor for so-called Th17 cells. Hence, reduced STAT3 signaling leads to recurrent infections. In contrast, increased STAT3 signaling leads to autoimmunity and cancer. In humans, STAT3 is expressed in two isoforms (the alpha and the beta variant) resulting from alternative splicing. The beta version lacks parts of the transactivation (TA) domain of the alpha version, and instead harbors seven unique amino acids. In addition to forming homodimers (and mixed alpha and beta homodimers), STAT3 forms heterodimers with STAT1 and STAT5. However, the conditions under which these heterodimers are being formed and their biological role are still unclear.

Therefore, in this project, DC4 will focus on addressing the following important topics related to STAT3 signaling biology: 1) study the importance of STAT1/3 splice variant expression on lymphocytes and mononuclear cells with and without stimulation and its impact on gene regulation and downstream gene expression; 2) analyze the impact of various stimuli on the heterodimerformation of STAT molecules and their consequences on gene regulation and downstream gene expression including chromatin accessibility; and 3) design and test a STAT3 gene therapy protocol with an expression cassette replacing the most frequent dominant-negative mutations in the gene STAT3.

Host institution: Universitätsklinikum Freiburg

Degree awarding institution: Albert-Ludwig Freiburg University, Germany

Supervisor: Dr. Bodo Grimbacher

Planned secondment(s): RFHMO (Stepensky/Schejter) to optimise sample preparation and recruitment, m10-m12 (2 months); IJC to perform epigenetic analyses, m13-m15 (2 months); IRB (Geiger) to perform proteomic analyses, m15-m17 EMBL (2 months); EMBL (Zaugg) to learn multi-omic data analysis, m22-m23 (1 month). Finally, the DC will do a secondment at qGenomics to learn cfDNA-seq analyses, m30-31 (1 month).

Doctoral Candidate 5: IKBKB deficiency

Rationale and Objectives: Inhibitor of nuclear factor kappa-B kinase subunit beta (IKK- β or IKK2), encoded by the gene IKBKB, is a critical subunit of the IKK kinase complex. By phosphorylation of the inhibitor of NF- κ B I κ B α it permits the activation of the canonical NF- κ B-mediated transcriptional program. Currently, over 20 patients with homozygous mutations in IKBKB have been described. Inborn errors in IKBKB lead to a profound combined immunodeficiency with early onset of a broad spectrum of infections. Together with Stepensky's group, we have identified a family with two siblings with deleterious homozygous deletion frameshift mutation. Both siblings suffered from mycobacterial infections and underwent HSCT after discovery of their profound immunodeficiency and died subsequent to secondary loss of graft. DC5 project's aims include: 1) to investigate the altered chromatin accessibility and transcriptome of B-, T cells and monocytes before and after respective activation in comparison to pre-existing data from patients with NFKB1 haploinsufficiency (manuscript in preparation) and data produced by the group of Bodo Grimbacher (DC4) in this consortium for A20 deficiency; 2) to confirm identified target genes by flow cytometry and functionally evaluate them in vitro; 3) to compare data to the results from the respective lymphocyte and monocyte populations from healthy controls treated with IKK- β inhibitors, a potential targeted therapy under current clinical evaluation. These experiments will allow us to explore the concordance of the respective findings between the natural knock-out and the drug-induced changes.

Host institution: Universitätsklinikum Freiburg

Degree awarding institution: Albert-Ludwig Freiburg University, Germany

Supervisor: Dr. Klaus Warnatz

Planned secondment(s): RFHMO (Stepensky/Schejter) to optimise sample preparation and recruitment, m6-m8 (2 months); IJC to perform epigenetic analyses, m10-m12 (2 months); EMBL (Zaug) to perform transcription factor network analyse, m20-21 (1 month); GRL (Vento-Tormo) to learn multi-omics data analysis, m21-22 (1 month). Finally, the DC will do a secondment at Alia Therapeutics to learn novel gene editing protocols, m25-27 (2 months).

Doctoral Candidate 6: Dissecting the role of dysfunctional telomeres to stem cell biology and immunity

Rationale and Objectives: Dyskeratosis congenita (DC) is a genetic inherited syndrome characterised by short telomeres. Telomerase is a specialised ribonucleoprotein complex composed of Telomerase Reverse Transcriptase (TERT), Telomerase RNA Component (TERC), and dyskerin, which stabilises telomerase complex. More than half of DC patients harbour mutations in telomere maintenance genes and immunodeficiencies and bone marrow failure (BMF) represent their main cause of mortality. Telomere attrition is one of the best-characterised mechanisms of cellular senescence. We hypothesise that telomere shortening triggers a DDR-dependent senescence in DC patients' BM-derived HSPC leading to severe BMF and



proinflammatory detrimental programs. DC6 will focus on the autosomal forms of the disease caused by TERC gene mutations, preferentially affecting paediatric patients. To that end, DC-like human HSPC will be generated by the (CRISPR)-Cas system. Our engineered DC human model will allow us to study the causes of HSPC premature exhaustion and hematopoietic dysfunctions, with a specific focus on: 1) exacerbated DDR (imaging/flow cytometry); 2) transcriptional and epigenetic changes (scRNAseq/histone marks); 3) proinflammatory phenotype (luminex assay) and will be used as a platform for the development of new therapies for DC patients. Functional experiments will include colony-forming assays in semisolid medium and long-term hematopoietic reconstitution by transplantation. Validation experiments will be performed in BM-derived HSPCs from DC patients obtained through a collaboration with the Gaslini Hospital in Genoa.

Host institution: Ospedale San Raffaele SRL

Degree awarding institution: Università Vita-Salute San Raffaele, Milan (Italy)

Supervisor: Dr. Rafaella Di Micco

Planned secondment(s): RFHMO (Stepensky/Schejter) to receive training on bone marrow sample processing, m10-m12 (2 months); IJC to utilize epigenetics to identify the molecular determinants of HSPC dysfunctions, m13-15 (2 months); GRL (Vento-Tormo), to identify the impact of the genetic inactivation in cellular and molecular phenotype by sc-omics, m20-22 (2 months). Finally, the DC will do a secondment at OneChain to learn the standards of genetic manipulation in the biotech sector, m30-31 (1 month).

Doctoral Candidate 7: Integrative framework for mapping the cell-type specific effects of mutations

Rationale and objectives: Mutations in transcription factors can have pleiotropic and unpredictable effects since they may impact many genes in many cell types, yet their direct target genes may differ in each cell type. The goal of this project is to generate a general framework for understanding the cell-type specific effect of any given patient-specific mutation in transcription factors studied by the consortium and elsewhere, with a focus on NFkB. The Zaugg group has recently developed a tool to generate cell-type specific gene regulatory networks¹¹, which are based on connecting transcription factors to enhancers to their target genes. In previous work, we have found that many TFs, including NFkB, regulate a very cell-type specific set of genes.

DC7's project description: To understand the impact of NFkB mutations in different immune cell types. For this, 1) to perform single cell RNA and ATAC-seq profiling in peripheral blood of 30 patients that harbour a mutation in NFkB along with 20 healthy donors. Using these data, we will devise a framework for generating gene regulatory networks based on inter-individual co-variation in RNA expression, transcription factor activity and enhancer accessibility, based on our previous work in bulk data¹¹. 2) Using this, to compare the regulon of NFkB across the different cell types and obtain a detailed map of the cell-type specific direct effects of NFkB mutations; 3)

using cell-type specific differential expression between the patients and healthy donors, our networks will identify transcription factors that are cooperating with NFkB to drive its cell-type specific effects. 4) The framework developed in this project will be used to integrate the multiomics data generated within and outside the consortium, to derive similar hypotheses for other mutations and to test the effect of corrections, and to interpret common genetic variants associated with immune disorders.

Host institution: European Molecular Biology Laboratory Heidelberg

Degree awarding institution: EMBL, Heidelberg, Germany

Supervisor: Dr. Judith Zaugg

Planned secondment(s): UKLFR (Warnatz) to receive training on patient sample processing, m10-12 (2 month); UKLFR (Grimbacher) to learn about NFkB signaling, m13-14 (1 month); GRL (Vento-Tormo) to establish a joint analysis framework, m15-18 (3months). Finally, the DC will do a secondment at qGenomics to learn how to process and analyse samples for cfATAC-seq, m25-27 (2 months).

Special selection process: the candidates interested in the EMBL International PhD programme (EIPP) will have to apply through the online IMMERGE website, and through the [EIPP webpage](#) well, in order to be formally admitted and allowed to enrol in the EIPP. Applicants are required to submit contact details of minimum 2 referees. The [2024 Winter Recruitment](#) is open until the 9th of October 2023 and the 2024 Summer Recruitment will open on the 25th January 2024 and will be available during the specific period in which the call is open. The selected candidate will have to undergo an additional row of interviews by the EMBL selection committee. Further information about the selection process will be provided to the selected candidates.

EMBL is an 'International European Research Organisations' (IERO), 'international organisations', or entities created under Union law, thus the researchers must not have spent more than 12 months in the 36 months immediately before their recruitment in the same appointing organisation.

Doctoral Candidate 8: Discerning the role of the NFkB pathway in inflammation, immunodeficiency and atopy through rare IEI

Rationale and Objectives: MALT1 serves as a scaffold protein for the CBM complex which is a key activator of the canonical NFkB pathway in the immune system. Additionally, it has a paracaspase function cleaving several proteins, including regulatory components of the NFkB pathway itself. Rare autosomal recessive defects in this gene cause type 2 inflammation, severe lymphoproliferation and combined immunodeficiency. CARMIL-2 deficiency is another AR defect with similar presentation to MALT1, including severe dermatitis and colitis as well as chronic and recurrent viral and bacterial infections. CARMIL2 mediates CARD11 activation downstream of CD28 co-signaling in lymphocytes. In this project, we aim to dissect the effect of MALT1 and CARMIL2 mutations in different immune cells and better understand the mechanism

underlying inflammation, atopy and lymphoproliferation in these defects. DC8's project aims are: 1) To compare samples from patients with MALT1 and CARMIL2 patients to healthy controls, as well as to samples of patients with NEMO and IKBKB deficiencies, which are associated primarily with immunodeficiency; 2) To stimulate patient PBMC's with various canonical and non-canonical NFkB stimulators and use sc-RNA-seq and ATAC-seq to dissect the transcriptional and epigenetic regulation of the NFkB pathway in different cell types; 3) To induce different defects of CARMIL2 and MALT1 in primary B- and T-cell lines, including null- and paracaspase-specific defects in the case of MALT1, and dissect the effect of these defects in the transcriptional and epigenetic levels post stimulation; 4) To generate induce pluripotent stem cells from patient cells and investigate the role of MALT1 and CARMIL2 in T- and B-cell differentiation. 5) In collaboration of Dr. Schwartz in RFHMO (GI), to induce organoids from gastrointestinal biopsies of CARMIL2 patients and dissect the role of CARMIL2 defects in inducing gut inflammation.

Host institution: Research Fund of the Hadassah Medical Organisation (RFHMO)

Degree awarding institution: Hebrew University in Jerusalem, Israel

Supervisor: Dr. Polina Stepensky

Planned secondment(s): GRL (Vento-Tormo) to utilize sc-RNAseq to identify the transcriptional profile of primary cells and cell lines upon stimulations, m15-m17 (2 months); qGenomics to identify epigenomic regulation of NFkB pathway through methylome analysis, m18-20 (2 months); EMBL (Zaugg) to create gene regulatory networks for patients with mutations in CARMIL2 and MALT1, m22-24 (2 months); IJC to determine impact of MALT1 and CARMIL2 defects on the epigenomic regulation of immune cells, m25-27 (2 months); and UKLFR (Warnatz) to analyse B-cell differentiation and function in CARMIL2 and MALT1 mutated cells, m28-30 (2 months).

Special selection process: the candidates selected for the RFHMO project will have to apply through the [Hebrew university PhD program](https://huji.ac.il) online application (huji.ac.il), in order to be formally admitted and allowed to enrol in the Hebrew University of Jerusalem. Details about special requirements for foreign students are described in the following [webpage](#). The candidates will have to undergo an additional screening and selection process, such as a personal interview by the University doctoral examination committee. Further information about the selection process will be provided to the selected candidates.

Doctoral Candidate 9: Exploring the role of JAK/STAT and NFkB signalling pathways in the onset of immune-related preeclampsia

Rationale and Objectives: Preeclampsia (PE) is a pregnancy-associated disorder characterised by the onset of hypertension that occurs after 20 weeks of gestation. It is a leading cause of maternal and perinatal mortality worldwide (15-20% in developed countries). An immune maladaptation of the mother to the foetus, involving the activity of the NFK-B and JAK/STAT

signalling pathways, might be underlying the incomplete trophoblast invasion and causing the early onset of PE. The importance of going deeper in understanding PE relies mainly on its unpredictable progression and the absence of effective treatment. This makes essential to develop preventive strategies allowing PE prediction/detection before the onset of its clinical manifestations. Consequently, DC9's project aims are: 1) Identify genetic variants in the JAK/STAT and NFkB signalling pathways that might predispose to the PE onset by exploring the unique cohort of primary immunodeficient patients available at the clinical laboratories. Novel genetic variants associated with PE will be identified by performing a custom WGS analysis against JAK/STAT- and NFkB-related genes. 2) Assess the potential of JAK/STAT and NFkB genetic variants to alter trophoblast formation in cellular models. Human induced pluripotent stem cells (hereafter hiPSCs), established from PB-MNCs of primary deficient women harbouring genetic variants that correlate with PE (identified in aim1), will be differentiated into trophoblast cells and the impact of the genetic variants evaluated by sc-RNAseq and sc-ATAC-seq. 3) Exploit chromatin accessibility at cell-free foetal DNA (cffDNA) as an early molecular biomarker for PE. Specific accessibility signatures in cffDNA allows for determining the PE-associated apoptosis in the trophoblast. Transcription factors (TFs) potentially binding to the PE-differential chromatin accessible regions will be identified. Finally, the predicted TFs will be compared to the genetic variants correlated with PE onset identified in the patient cohort to validate the PE prediction based on cell-free foetal DNA.

Host institution: Quantitative Genomics Medicine Laboratories SL

Degree awarding institution: University Pompeu Fabra (UPF), Barcelona Spain

Supervisors: Dr. Lluís Armengol/Dr. Jairo Rodríguez (qGenomics)

Planned secondment(s): RFHMO (Stepensky/Schejter) (m10-m11 -1month-) and UKLFR (m11-m12 -1month-) to optimise sample preparation and recruitment; GRL (Vento-Tormo) to profile by sc-omics the impact of specific mutations on trophoblast cells, m25-27 (2 months); and EMBL (Zaugg) to infer the footprints of specific transcription factors in the cfATAC-seq datasets generated, m28-m30 (2 months).

Doctoral Candidate 10: Correction of mutations associated with primary immunodeficiencies using novel and engineered CRISPR tools

Objectives: Primary immunodeficiencies are a group of highly heterogeneous disorders, characterized by an increased susceptibility to infections, coupled with a higher risk of developing autoimmune diseases or cancer. Common variable immunodeficiency disorders (CVID) due to mutations in CTLA-4 have been particularly linked to the development of autoimmunity. Dominant negative mutations in STAT3, which have been associated to Hyper-IgE syndrome (HIES), are also of interest in the gene editing field since its impaired function has been related to immunodeficiency as well as tumorigenesis. Alia Therapeutics has developed a discovery and engineering platform for the isolation of new CRISPR nucleases with high activity and specificity.

DC10's goal is to apply our gene editing technology to target monogenic primary immunodeficiencies to identify potential therapeutic approaches for the treatment of such disorders. Therefore, Alia's editing technology will be applied to edit CTLA-4 or STAT3 dominant-negative mutated cells. The project will be split in four phases: 1) Identify undescribed nucleases from our dataset which best fit the above-mentioned targets. 2) Develop a tailored editing strategy for the two targets: a gene replacement strategy (knock-in) for CTLA-4 deficiency will be evaluated; and a classical allele-specific NHEJ-strategy for STAT3 mutant will be tested to downregulate its expression. 3) Validate in vitro the efficacy (editing, mRNA and protein downregulation or upregulation) and safety (genome wide off-targets, allele-specificity if needed) of the editing approaches; 4) Evaluate the functional recovery upon gene correction by exploiting available mouse models.

Host institution: Alia Therapeutics srl

Degree awarding institution: University of Trento, Italy

Supervisor: Dr. Antonio Casini

Planned secondment(s): IJC (Ballestar) to learn in vitro differentiation protocols of immune cells, m10-m12 (2 months); OSR (Di Micco) to learn in vivo differentiation assays, m13-15 (2 months); IRB (Geiger) to learn T cell biology, m16-17 (1 month); and GRL (Vento-Tormo) to perform and analyse sc-omics on corrected cells, m25-m28 (3 month).

Doctoral Candidate 11: Identifying novel regulators underlying Tregopathies

Objectives: Tregs play a particularly critical role in restraining immune responses to self and foreign antigens and their associated inflammation. "Tregopathies" collectively manifest in multiorgan autoimmunity and are caused by loss-of-function mutations in FOXP3, CD25, CTLA4, LRBA, BACH2 and gain-of-function mutations in STAT3. The functional roles of these genes in Tregs have been well studied, which facilitated the identification of mutations in these genes as drivers of "Tregopathies". However, the spectrum of mechanisms by which Tregs suppress effector T cells and other immune cells is still not fully understood. DC11's project aims include: 1) to perform a genetic screen coupled with an in vitro Treg suppression assay to identify the full spectrum of genes that are involved in Treg-mediated immune suppression; 2) to leverage an arrayed CRISPR/Cas9 knockout library to ablate genes in primary human Tregs; 3) to co-culture in 384 well plates with donor-matched T effector cells in the presence of an activation stimulus. The suppressive effect of Tregs on T effector cell proliferation will be analysed by high throughput flow cytometry. Tregs that lost the ability to suppress effector cells will be analysed and the genes that are ablated in these cells will be considered as hits for in-depth follow up analyses. A comprehensive understanding of genes involved in Treg function will expedite the identification of new inborn errors that cause dysfunction of Tregs. This will be essential to accelerate diagnoses and the design of new targeted therapies to alleviate diseases symptoms or curative approaches that correct the mutations in the genes.

Host institution: Fondazione per l'Istituto di Ricerca in Biomedicina

Degree awarding institution: Università della Svizzera italiana (Lugano), Switzerland

Supervisor: Dr. Roger Geiger

Planned secondment(s): UKLFR (Grimbacher) to study cells from patients with Tregopathies, m10-13 (3 month); and Alia Therapeutics to learn the standards of genetic manipulation in the biotech industry, m25-27 (2 month).

Doctoral Candidate 12: Uncovering alterations during hematopoietic differentiation in immune monogenic syndromes

Rationale and Objectives: A subgroup of monogenic IEI patients is characterised by mutations in JAK-STAT signalling proteins, which comprise several kinases and transcription factors involved in inflammation. Gain-of-function (GOF) and loss-of-function (LOF) mutations are usually associated with life threatening immune dysregulation leading to an exacerbated inflammatory response and autoimmunity. DC12's project aims are: 1) To functionally characterise the germline variants in the JAK-STAT pathway (15-20 samples, both pediatric and adult) in steady state and following activation, using single cell omics approaches (scRNASeq, scATACseq) and on peripheral blood bone marrow and biopsies; 2) To model JAK-STAT mutations by differentiating human induced pluripotent stem cells (hiPSC) carrying JAK-STAT mutations into multiple immune cell lineages 3) To investigate the effects of different JAK-STAT inhibitors in the differentiation and cell-cell crosstalk using tools such as CellPhoneDB10 and in vitro modelling.

Host institution: Genome Research Limited (SANGER Institute)

Degree awarding institution: University of Cambridge, UK

Supervisor: Dr. Roser Vento-Tormo

Secondments: UKLFR to receive training on various immunological methods, m10-m13 (3 months); Finally, DC12 will learn how to commercialise the output from his/her (epi)genetic findings by doing secondments at qGenomics (m26-m27 -1 month-) and EpiQMax (m27-m28 -1 month-).

Special selection process: The selected candidate will have to undergo an additional row of interviews by the Sanger selection committee. Further information about the selection process will be provided to the selected candidates.

REQUIREMENTS:

Eligibility criteria

We welcome applications from Doctoral Candidates (DCs) from any country and nationality, fulfilling the following criteria:

- Eligible candidates must not have a doctoral degree at the date of their recruitment.
- Eligible candidates must not have resided or carried out their main activity (work, studies, etc.) in the country of the recruiting organisation for more than 12 months in the 36 months immediately before their recruitment date (i.e. the starting date indicated in the employment contract/equivalent direct contract).
- Eligible candidates must have a master's degree relevant to the chosen position (including biology, medicine, biochemistry, bioinformatics or a related discipline, depending on each PhD project) or its equivalent that would entitle them to a doctorate one month before the labor contract starts, or must hold an official university qualification from a country of the European Higher Education Area with a minimum of 300 ECTS of official university studies.

Successful candidates must have a high level of proficiency in written and spoken English, which will be assessed with the motivation letter and the interview, respectively.

ADDITIONAL INFORMATION:

Application and selection process

The application will be done through an online application platform to be found on the IMMERGE website: www.immergeproject.eu. Applications must be in English. Each applicant may apply to a **maximum of three individual research projects**.

The entire eligibility and recruitment process will be led by IMMERGE Recruitment Board (RB) and supervised by the Supervisory Board (SB). Eligible applications will be ranked based on CVs and merits by selection committees for individual DC positions, created by the supervisor, co-supervisor, and a member of IMMERGE Training Committee.

IMMERGE RB will evaluate the following criteria:

- the CV: Academic merit, experience, mobility, and publication track-record.
- Motivation letter: quality of writing, match of interest, strengths.

The 3 best candidates for each position will be invited to an online recruitment workshop in January (date to be confirmed) where the final candidates will be selected.

Applicants with a positive evaluation, but not selected, will be included on a reserve list to cover eventual future positions and might be contacted at a later stage.



Timeline (Tentative)

- Application deadline: 3rd December 2023
- Announcement of preselection results and call for interviews: 22nd December 2023
- Recruitment workshop: mid January 2024. The three top candidates per position will be invited to the online interviews. Full details regarding the interview process will be sent to invited candidates during the arrangement of interviews.
- Communication of the final results: January 2024
- Tentative start of the fellowship: March-September 2024

Benefits

- 3-year full-time employment contract (salary depends on the country of the recruitment considering both the local and MSCA DN regulations for DCs and their family status at the time of the recruitment, that is the starting date of the contract).
- Enrolment in a PhD programme.
- Shared research and innovative multidisciplinary and multisectoral training by experts and experienced trainers from two sectors (academia and industry) and two research environments (clinic and basic).
- A structured training programme consisting of soft skill courses, targeted workshops, retreats, social events, and networking.
- Secondments at other institutions within the IMMERGE consortium.
- Gaining experience abroad.
- Opportunities for participation in national and international meetings.
- Enlarged professional network and improved future scientific career perspective in academia and the private sector.

For further information on the IMMERGE and the application process, please visit www.immergeproject.eu.

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